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IS A BEE ATTRACTED TO CLOVER BLOSSOMS BY ODOR?

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Introduction

Since Darwin's (6) experimental proof, that cross fertilization has decided advantages over self fertilization, pollination by honey-bee has become a common practice. Hendrickson (7) showed that insects may be necessary for the application of the pollen, even of some self-fertilized varieties. Alderman (1) and Auchter (2) showed increase of fruit setting by bees.

Most entomologists, such as Barrows (3), Lovell (10) and McIndoo (14) have advocated that bees respond to odor. Others as Bulman (4), Lovell (9) and Clements and Long (5), attribute the visit of honey-bees to an attractive color of flowers. Many botanists agree with the latter view. For instance Holman and Robbins say "In typical flowers, the petals attract insects" (8). In fact they think, that in the evolution of flowers, color changes have taken place because of their symbiotic relationship with the bee or other pollinating insects.

In many experiments, at least those in which oligotropism has been studied, some artificial odor or odors (not of the same species of flowers) have been used. Thus one of the main points, namely, whether the bee prefers a specific odor or color in a particular flower has been neglected. Yet this problem is not only of theoretical importance but also of practical significance as pointed out by Malhotra (11).

The lack of specific color and odor information in this connection seems to be for want of a reliable method of securing a reasonable amount of odorous material of a type of flower to be studied and the use of such a product under strictly analytical conditions. An attempt has been made to develop such a method. It may be capable of not only studying the odor responses in bees but may also yield similar information with reference to other pollinating insects.

Methods and Materials

Six hives of 100 black or German honey-bees were secured. Six tents $5 \times 7 \times 8$ feet were prepared by using a single layer of cheese-cloth and wooden frames. A bee hive was placed in each tent, which was sealed to the ground.

A red clover field with abundant pollen was selected. Twenty grams of pollen and about 150 grams of flowers were collected in air-tight phials. It was recognized previously, as stated by Malhotra (12) that odor in flowers is due to an ester or esters. About 5–7 cc. of these esters were separated by fractional distillation (15). This product was further treated in order to obtain a more concentrated odor of clover flowers.

A filter-paper thimble, as used in the Soxlet extraction apparatus, was prepared. After putting three grams of pollen in, it was sealed. The use of a special thimble was necessary, since it was found by preliminary trials that any type of manufactured thimble, at present used in chemical laboratories, would be unsatisfactory for pollen extracting, first, because pollen may be easily damaged, and second, because it is very light and might float in an open thimble as soon as the condensed drops of the medium fall directly on it. The pollen was deprived of its essential oils (odorous material) by extracting it with petrolic ether (range 32° C.) for ten hours (at a special adjusted electric hot plate) by means of the Soxlet extraction method (13). Ether extracted pollen was divided into ten lots of about half a gram each.

The remaining pollen was weighed very accurately (four decimal places) in lots of about a half gram each. Microscopic slides were thoroughly cleaned, dried at 40° C., cooled in a desic-

cator and accurately weighed. About four drops of honey (secured from bees of the same species and out of the same clover field) were placed on each slide. This weighed honey was entirely covered by pollen.

Treated and untreated pollen was divided into various lots. Lot A contained normal pollen. Pollen of lot B was deprived of odor and color. To pollen of lot C, two drops of essential oil extracted from clover flowers were added. Pollen of lot D was treated similarly to lot B, except that it was colored as normal pollen. Two slides of each lot were placed in each tent for a week. The experiment was repeated four times for a month.

The slides with the unused material were dried, cooled and weighed as before. Loss of pollen was figured on a percentage basis. It was assumed, that the loss of pollen weight was due to its being carried away by bees and that the more frequent the visits, the greater the loss. Difference in various lots, with respect to weight loss, may be attributed to difference in attraction of flowers.

Results in repeated experiments appear to be very similar within various lots. However, for economy of space, the data for only one representative experiment will be reported in this paper.

Presentation of Data and Summary

Data presented in Table I seems to indicate that pollen, to which odorous material almost similar to that found in natural

TABLE I

DATA SHOWING PERCENTAGE LOSS OF POLLEN DUE TO VISITS OF BEES

Lot No.	Description of Pollen Treatment	Percentage Loss of Pollen	Observational Remarks
A	Normal pollen	58.00	Visits very frequent
B	Pollen minus color and odorous material	11.00	Frequently visited
C	Pollen plus odorous material	66.7	Very few visited
D	Pollen like B plus color of pollen	13.6	Few visits

clover flowers was added, was most favorable for attracting bees, as indicated by the percentage loss of pollen under the conditions of this experiment. Normal pollen (Lot A) attracted little less than Lot C. Pollen artificially colored but deprived of odor (Lot D) attracted bees little better than pollen in which both color and odor of a natural clover flower (Lot B) were absent. However, the difference between lots B and D is so small that it can fall within the range of an experimental error.

In general, it would seem from the data presented above, that this race of bees is primarily attracted to clover flowers due to odor rather than color, at least during the course of this study. This may or may not be the only mechanism in all or most cases, since only one kind of flower and a single species of bees have been used. This study is being extended, and may show any divergence of these insects in this respect. So far as this study goes, it seems that odor attracts bees to a larger extent than color.

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